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## Functional diversity in resource use by fungi

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**Abstract.** Fungi influence nutrient cycling in terrestrial ecosystems, as they are major regulators of decomposition and soil respiration. However, little is known about the substrate preferences of individual fungal species outside of laboratory culture studies. If active fungi differ in their substrate preferences in situ, then changes in fungal diversity due to global change may dramatically influence nutrient cycling in ecosystems. To test the responses of individual fungal taxa to specific substrates, we used a nucleotide-analogue procedure in the boreal forest of Alaska (USA). Specifically, we added four organic N compounds commonly found in plant litter (arginine, glutamate, lignocellulose, and tannin–protein) to litterbags filled with decomposed leaf litter (black spruce and aspen) and assessed the responses of active fungal species using qPCR (quantitative polymerase chain reaction), oligonucleotide fingerprinting of rRNA genes, and sequencing. We also compared the sequences from our experiment with a concurrent warming experiment to see if active fungi that targeted more recalcitrant compounds would respond more positively to soil warming. We found that individual fungal taxa responded differently to substrate additions and that active fungal communities were different across litter types (spruce vs. aspen). Active fungi that targeted lignocellulose also responded positively to experimental warming. Additionally, resource-use patterns in different fungal taxa were genetically correlated, suggesting that it may be possible to predict the ecological function of active fungal communities based on genetic information. Together, these results imply that fungi are functionally diverse and that reductions in fungal diversity may have consequences for ecosystem functioning.

**Key words:** Alaska, USA; boreal forest; fungi; global change; nitrogen; nutrient cycling; resource partitioning; substrate use; warming.

### INTRODUCTION

Fungi, along with other soil organisms, decompose dead plant material and therefore influence dynamics of C and N in soils. Moreover, fungal communities frequently shift under global change (Horton and Bruns 2001, Treseder 2005, Allison and Martiny 2008). Molecular analyses have documented decreases in the diversity of decomposer fungi following N addition (simulating anthropogenic N deposition) in the Alaskan boreal forest (Allison et al. 2007, 2008). In other systems, the diversity of ectomycorrhizal fungi, a functional group of fungi that form mutualisms with plants, frequently decreases following N fertilization or deposition (Lilleskov et al. 2002, Frey et al. 2004). Conversely, experimental warming raises decomposer

fungal diversity in Alaskan boreal forest (Allison and Treseder 2008), and in Harvard Forest (Hanson et al. 2008). The consequences of fungal diversity for nutrient cycling are still unknown. However, fungi are poised to play a regulatory role in carbon sequestration and nutrient mineralization under global change.

If fungal groups differ markedly in substrate preference in situ, then changes in fungal diversity may influence nutrient cycling rates. Because different suites of enzymes are required to assimilate different substrates, and because enzyme construction is costly in terms of energy (Stouthammer 1973) and N (Allison and Vitousek 2005), individual fungal groups may produce enzymes that target only a specific subset of available substrates (Schimel et al. 2004). Resource partitioning may be one mechanism for niche separation or substrate use preferences. Laboratory and greenhouse-based studies indicate that fungal groups (including species and genetic varieties) can differ in growth on diverse C sources (Durall et al. 1994, Tuomela et al. 2002) and N forms (Abuzinadah and Read 1986, Gebauer and Taylor 1999). However, few studies have been conducted in natural ecosystems, so extrapolations of these traits to fungi in natural systems should be performed only with

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caution. Methodological limitations have been major impediments to conducting studies of fungi in natural ecosystems. Over the last several decades, the molecular revolution has enabled microbial ecologists to make significant strides in understanding the enormous complexity and diversity of microbes in natural habitats. Nonetheless, each molecular technique used to identify the taxonomic and functional diversity of microbes has limitations and biases. Therefore, full integration of artificial experiments to ecosystem processes has not yet been accomplished.

One potential way to better link greenhouse and laboratory studies to natural systems is to explore the links between genetic and phenotypic relatedness of fungi. If a genetically related group of fungi displays similar substrate-use preferences, then we may be able to infer the functions of other active fungal communities, even if we only have sequence data. Genetic and phenotypic similarities are often correlated in nonmicrobial systems (Damerval et al. 1987, Scharloo 1991, Villani et al. 1992), and this principle is frequently invoked in environmental studies of microbial communities (Pace 1997). For example, a standard practice is to compare DNA sequences obtained from environmental samples to the published sequences of cultured, described microbes. Ecological functions of the unidentified species are then inferred from those of their closest relatives. However, intraspecific variation in nutrient use can be as great as variation among species in the laboratory (Cairney 1999). If this trend occurs in natural systems as well, our ability to predict function based on genetics may be limited.

We examined these issues in Alaskan boreal forest. This region is experiencing anthropogenic warming and proliferation of forest fires (Kasischke and Stocks 2000), which alter the quantity and quality of organic material available for decomposition (Bergner et al. 2004, Treseder et al. 2004). We hypothesized that fungal taxa should vary in the degree to which they use different forms of organic N, and as a result, fungal community composition would shift in response to the addition of these substrates (Hypothesis 1). Moreover, we hypothesized that fungal taxa would be negatively related with one another in terms of relative abundance, due to niche separation via resource partitioning (Hypothesis 2). We also hypothesized that leaf litter type (newly senesced leaves/needles) would influence fungal community composition, owing to differences in litter chemistry (Hypothesis 3). Fungi that were closely related genetically were expected to display relatively similar patterns of substrate use (Hypothesis 4). Finally, we used DNA sequences obtained from a concurrent warming experiment in this site to determine if substrate use by taxa was related to temperature sensitivity. We predicted that taxa that targeted recalcitrant N compounds would respond more positively to warming than would those that did not, because warmer temperatures would be more critical in overcoming potentially high activation

energies associated with recalcitrant compounds (Davidson and Janssens 2006) (Hypothesis 5).

## METHODS

### *Field sites*

Our study site was located in an upland boreal ecosystem near Delta Junction, Alaska, USA (63°55' N, 145°44' W) detailed in Treseder et al. (2004). Briefly, this ecosystem is an 85-year-old, mature forest dominated by *Picea mariana* (black spruce) with an understory of lichens, mosses, and shrubs. The mean annual temperature is  $-2^{\circ}\text{C}$ ; the precipitation rate, 303 mm/yr (data available online).<sup>7</sup> Permafrost is discontinuous in this area and is not present in the site. Soils are well-drained. The growing season extends from bud break in mid-May to leaf fall in mid-September.

### *Experimental design and nucleotide analogue labeling*

Senescent leaves were plucked directly from black spruce (*Picea mariana*) and quaking aspen (*Populus tremuloides*) in this site in September 2002, air dried, and stored at  $20^{\circ}\text{C}$ . Litter was placed in 1-mm mesh bags (4 g litter/bag), sterilized in a Cs-137 gamma irradiator overnight (2.5 total Mrad), and then embedded in the organic layer at the site in September 2005. Litterbags contained either black spruce or aspen litter, with three replicates per litter type. Litterbags were collected in September 2006, placed immediately on ice, and then transported directly to University of California–Irvine. Nucleotide analogue labeling (below) and substrate additions were performed within 48 hours of litterbag collection.

For substrate additions, we used four organic N compounds: two common N storage compounds in plants (arginine and glutamate), protein complexed with condensed tannins, and lignocellulose. These substrates typically comprise a significant portion of plant litter N (Stevenson 1994). Ostensibly, fungi degrade lignocellulose to gain access to N-containing compounds that are protected by or complexed with lignin (e.g., Haselwandter et al. 1990). From each litterbag, five 15-mg subsamples of litter were analyzed separately for each substrate addition. We added a total of 100  $\mu\text{g}$  N to each 15 mg subsample of litter from each litterbag. The N source was dissolved in 130  $\mu\text{L/g}$  of litter (wet mass) in the form of arginine, asparagine, glutamate, protein complexed with condensed tannins, or purified lignocellulose (assuming that lignocellulose is 0.2% N by mass (Sanderson and Wedin 1989). To prepare lignocellulose, we followed a modified version of protocols from Crawford and Crawford (1976) and Crawford et al. (1977) using needles from *Pinus canariensis* (see Appendix A). We generated tannin–protein complexes by dissolving 1 g tannic acid (Fisher Scientific, Waltham, Massachusetts, USA, S80215) in 1-L deionized water to

<sup>7</sup> (<http://weather.noaa.gov/>)

create a tannin solution (see Appendix A). Each N-containing solution was applied to the top of litter and not mixed in.

Bromodeoxyuridine (BrdU), which is an analogue for the thymidine nucleotide, was introduced with substrates simultaneously following the protocol of Hanson et al. (2008). This nucleotide analogue allowed us to identify microbes that proliferated in response to additions of specific compounds (Borneman 1999). One control (nucleotide analogue with no substrate added) was analyzed for each litterbag. Thus, one subsample from each litterbag was given glutamate + BrdU, arginine + BrdU, tannin-protein + BrdU, lignocellulose + BrdU, and a control (BrdU with no N source), for a total of 30 assays.

Incubations were conducted in weigh boats for 48 hours at 22°C in the dark. The fungi whose growth was stimulated by the substrate additions should incorporate the BrdU into their newly synthesized DNA. Bulk DNA was extracted from soil samples using a bead-beating method (Yeates and Gillings 1998). Anti-BrdU antibodies attached to magnetic beads were used to isolate BrdU-labeled DNA. The BrdU-containing DNA was then analyzed by oligonucleotide fingerprinting of rRNA genes (OFRG) and nucleotide sequence analysis (see *Methods: Oligonucleotide fingerprinting* . . .). The 48-hour incubation time was determined through a series of preliminary trials; we found that after 48 hours, novel BrdU-labeled taxa became evident. We selected this incubation time as a conservative choice to avoid issues related to cross-feeding.

#### *Oligonucleotide fingerprinting of rRNA genes and qPCR*

Oligonucleotide fingerprinting of rRNA genes (OFRG) was used to characterize the identity of fungal groups in the DNA samples following the procedures of Bent et al. (2006). OFRG sorts rRNA gene clones at the genus- to species-level of resolution (Valinsky et al. 2002a, b, Yin et al. 2003). Briefly, BrdU-labeled fungal small-subunit rRNA genes were PCR amplified using universal fungal primers nu-SSU-0817-5' and nu-SSU-1196-3' (Borneman and Hartin 2000). These rRNA gene fragments were then cloned into a plasmid vector, transformed into *E. coli* cells, and subject to probe hybridization and OFRG fingerprint analysis (see Appendix A). Approximately 384 rRNA gene clones were analyzed for each of the 30 DNA samples.

We used quantitative PCR (qPCR) on total DNA to determine total fungal and bacterial abundance. Amplifications were performed using the fungal-specific (partial 18S rRNA gene) primers nu-SSU-0817-5' and nu-SSU-1196-3' (Borneman and Hartin 2000) and the bacterial-specific (partial 16S rRNA gene) primers Eub338 and Eub518 (Fierer et al. 2005; Appendix A).

#### *Comparison with sequences from warming experiment*

Allison and Treseder (2008) conducted a manipulative warming experiment in this field site. Briefly, five

replicates of 2.5 × 2.5 m control and greenhouse-warmed plots were maintained during the growing seasons of 2005–2007, and soils were collected in August 2006 for DNA extraction and fungal community analysis by cloning and sequencing. The authors used the same primers to sequence the same partial 18S rRNA gene region as for the current study. A total of 870 clones were sequenced. Sequences of the current study were aligned with sequences from the warming study using ClustalW version 1.83 (Chenna et al. 2003). To determine if the sequences from the current study were also present in the warming treatments, we employed DOTUR (Schloss and Handelsman 2005) and used a 97% sequence similarity cut-off for OTU groupings (based on a furthest-neighbor algorithm).

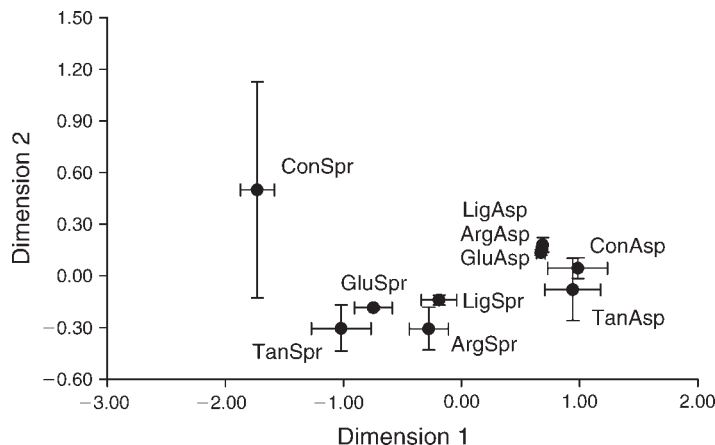
#### *Statistics*

To test for effects of substrate addition on fungal community composition, we performed split-split plot ANOVAs. The dependent variable was relative abundance (ranked); the main factor, litter type; the split factor, substrate addition; and the sub-split factor, taxon. We also conducted split plot analyses of variance (ANOVAs) on qPCR results, with fungal DNA abundance or fungal-to-bacterial ratios (ranked) as the dependent variables, litter type as the main factor, and substrate addition treatment as the split factor (Milliken and Johnson 2002). To measure the extent to which individual taxa responded to each substrate addition (or litter type), we calculated Cohen's *d* effect sizes (Cohen 1992; Appendix A). We also analyzed the effect of substrate addition and litter type on phylogenetic community structure using the PHYLOCOM software package (Webb and Donoghue 2005; Appendix A). To visualize similarity of fungal community composition, we used nonmetric multidimensional scaling (NMDS) on relative abundance data, by using the Kruskal method with monotonic scaling. These analyses were performed with SYSTAT (SPSS 2002).

To test for negative relationships among fungal taxa, we used EcoSim (Gotelli and Entsminger 2001) to calculate one C score for samples from all treatments and another for samples from the controls only. The analyses were performed with 5000 iterations, fixed row sums, and a sequential swap algorithm. C scores indicate the degree to which taxa co-occur; higher C scores indicate less co-occurrence among taxa, on average (Stone and Roberts 1990).

To determine if closely related taxa displayed relatively similar substrate use profiles, we performed a Mantel nonparametric test with 10 000 permutations (Mantel 1967), comparing genetic distance (proportion of unshared base pairs) and phenotypic distance (Euclidean distance of the effect sizes of each substrate or litter type (Liedoff 1999; Appendix A)). We used Pearson correlations to test for relationships between effect sizes of substrate use (from the current study) and warming (from Allison and Treseder [2008]). We also

FIG. 1. Nonmetric multidimensional scaling plot of fungal community composition (Euclidean distance of the response ratios for different fungi) in substrate addition and litter treatments (spruce and aspen) in an upland boreal ecosystem near Delta Junction, Alaska, USA. Symbols represent means  $\pm$  SE of three samples. Samples that are closer together harbored similar active fungal communities, compared to others. Key to abbreviations: Con, control; Arg, arginine; Glu, glutamate; Lig, lignocellulose; Tan, tannin-protein complexes; Spr, spruce; Asp, aspen.



used Pearson correlations to generate a correlation matrix of substrate effect sizes within taxa.

## RESULTS

### *Response to substrate additions and litter type*

Oligonucleotide fingerprinting of rRNA genes (OFRG) identified 17 dominant taxa, 16 of which could be sequenced (Appendix B). Pairwise identities of the rRNA gene sequences within the taxa ranged from 95.7% for Ascomycota 3 to 99.9% for Tremellomycete 2.

The 17 most abundant taxa were used for additional analyses (Appendix B). The selected taxa represented a broad distribution of phylogenetic groups, including yeasts, other ascomycetes, and basidiomycetes (Appendix B). In support of Hypotheses 1 and 3, these groups varied in their responses to substrate additions, as indicated by a significant  $\text{taxon} \times \text{substrate addition}$  interaction (Appendix B;  $F_{64, 256} = 1.827$ ,  $P = 0.001$ ); and in their distributions on aspen vs. black spruce litter, as indicated by a significant  $\text{taxon} \times \text{litter type}$  interaction ( $F_{16, 256} = 12.450$ ,  $P < 0.001$ ). Nonmetric multidimensional scaling separated active fungal communities strongly between litter types, with spruce samples clustering in lower values of Dimension 1 and aspen samples in upper values (Fig. 1). In addition, active fungal communities differed among substrate addition treatments (Fig. 1). The final stress was 0.053.

The phylogenetic structure of active fungal communities was affected by both litter type and the addition of some substrates (Fig. 2). Fungi in aspen litter were significantly clustered with the addition of glutamate ( $P = 0.04$ ), lignocellulose ( $P = 0.037$ ), and tannin-protein ( $P = 0.027$ ). Fungi in spruce litter, by contrast, were overdispersed in response to the addition of glutamate ( $P = 0.048$ ) and lignocellulose ( $P = 0.022$ ). The total phylogenetic diversity as measured by the “*rao*” command in PHYLOCOM was unaffected by substrate addition within and across litter types. However, phylogenetic diversity in spruce litter was significantly greater than in aspen litter (17.15 vs. 11.57;  $P = 0.001$ ). Phylogenetic diversity is calculated as the sum of the

lengths of all the branches of the minimal subtree connecting these taxa, so the actual values can be considered distinct lineages.

Six taxa were significantly affected by substrate treatment (Appendix B). Altogether, the taxa with significant responses to substrates represented a broad range of phylogenies, and they included rare as well as common fungi. Glutamate, arginine, and lignocellulose generated responses in multiple taxa. In contrast, just

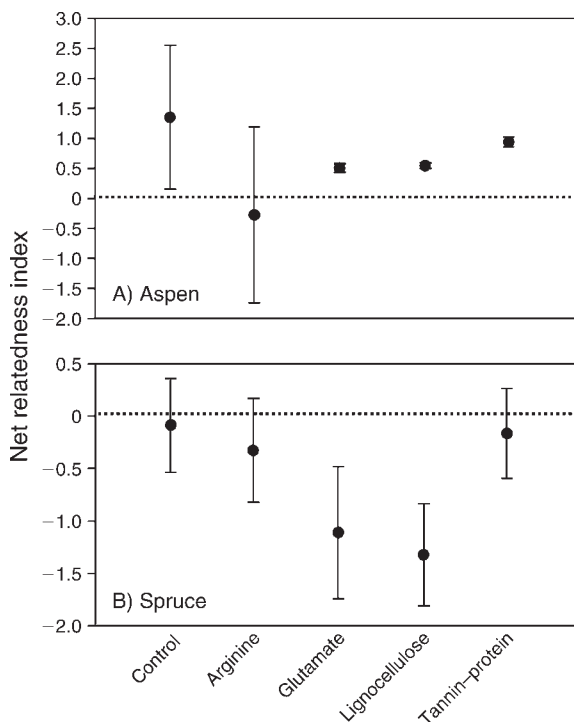


FIG. 2. Net relatedness index (NRI; mean  $\pm$  SE) for fungi detected after substrate additions (x-axis) within (A) aspen and (B) spruce litter. Positive NRI values indicate phylogenetic clustering of taxa that responded to the substrate additions, negative values indicate overdispersion, and values of zero indicate no pattern. Dotted lines were added across the zero intercept for clarity.



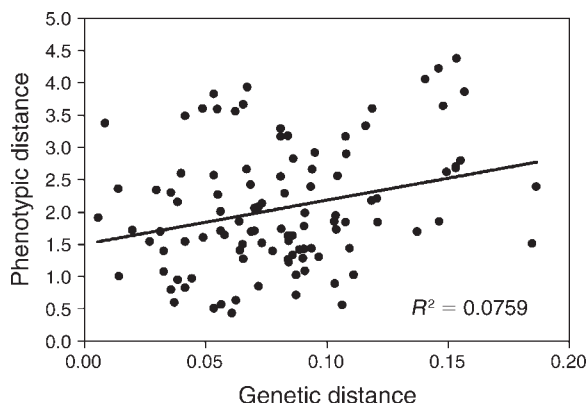


FIG. 3. Genetic vs. phenotypic relationships of fungal taxa. Phenotype is defined as the degree to which taxa responded to organic N compounds. A Mantel test indicated a significant positive correlation between phenotypic distance (difference between Cohen's  $d$  effect sizes) and genetic distance (the proportion of unshared base pairs), indicating that closer relatives tended to display similar substrate use profiles. Symbols represent comparisons between pairs of taxa, and the line represents the best-fit regression.

one taxon, *Sistotrema* sp.2, responded significantly to tannin–protein complexes; its response was negative and was only evident on black spruce litter. There were no significant interactions between substrate addition and litter type ( $F_{4,16} = 0.735$ ,  $P = 0.581$ ) or among taxa, substrate addition, and litter type ( $F_{64,256} = 1.167$ ,  $P = 0.203$ ).

The fungal community displayed less co-occurrence among taxa than expected by chance, as indicated by relatively high C scores. This pattern was evident across all treatments (standardized effect size = 2.46,  $P = 0.016$ ) and also within controls (standardized effect size = 2.04,  $P = 0.045$ ). These results supported Hypothesis 2, which stated that fungal taxa would be negatively related with one another in terms of relative abundance, due to niche separation via resource partitioning.

Taxa that were more closely related to one another also displayed relatively similar substrate use patterns. This finding supported Hypothesis 4. Specifically, genetic distance was significantly positively correlated with phenotypic distance (Euclidean distance of the

degree of response to each substrate; Fig. 3;  $g = 2.017$ ,  $P = 0.030$ ). In contrast, we found no significant relationship between genetic distance and differences in degree of response to litter type ( $g = 0.643$ ,  $P = 0.293$ ). Genetic distance between pairs of taxa ranged from 0.006 to 0.186.

In general, taxa that responded to a given substrate were also likely to respond to one of the other three substrates. For example, Cohen's  $d$  values for arginine were strongly correlated with those for glutamate across taxa (Appendix B;  $r = 0.868$ ,  $P < 0.001$ ). In fact, responses to all substrates were significantly correlated with one another ( $r \geq 0.678$ ,  $P \leq 0.003$ ) except for lignocellulose vs. tannin–protein complexes, which were marginally significantly correlated ( $r = 0.388$ ,  $P = 0.062$ ). We found no significant relationships, however, between responses to litter type and responses to any substrate addition ( $|r| \leq 0.169$ ).

Quantitative PCR analysis revealed that fungi were significantly more abundant on aspen litter than on black spruce litter (Appendix B;  $F_{1,4} = 17.362$ ,  $P = 0.014$ ). Substrate additions did not significantly alter fungal abundance ( $F_{4,16} = 1.144$ ,  $P = 0.371$ ), and there were no significant interactions between substrate addition treatments and litter type ( $F_{4,16} = 1.391$ ,  $P = 0.281$ ). Likewise, fungal:bacterial ratios differed significantly between litter types ( $F_{1,4} = 42.125$ ,  $P = 0.003$ ) and among substrate additions ( $F_{4,16} = 4.539$ ,  $P = 0.012$ ), but with no significant interaction between litter type and substrate ( $F_{4,16} = 2.883$ ,  $P = 0.951$ ).

#### Comparison with sequences from warming experiment

Nine taxa from the current study were also detected in the warming study (Table 1). However, in the OFRG analysis, some of these taxa had been sorted at a level of resolution  $>97\%$  sequence identity. As a result, when we aligned and grouped our sequences with the warming taxa (operational taxonomic units or OTUs) at 97% sequence identity, some of the OFRG taxa became grouped together (Table 1). Responses to experimental warming were correlated with responses to lignocellulose additions among taxa common to both experiments (Fig. 4;  $r = 0.874$ ,  $P = 0.026$ ). In other words, putative lignocellulose users tended to proliferate under experi-

TABLE 1. Effect sizes (Cohen's  $d$ ) for taxa common to the current study (a fungal community in an upland boreal ecosystem near Delta Junction, Alaska, USA) and a concurrent warming experiment.

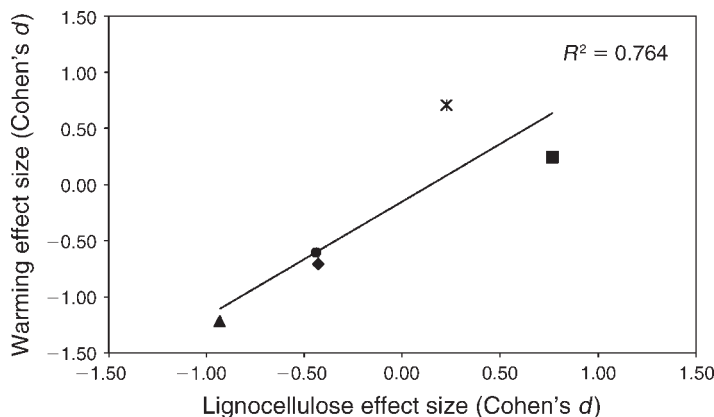
| Taxon                                   | Arginine | Glutamate | Lignocellulose | Tannin–protein | Warming† |
|---|----------|-----------|----------------|----------------|----------|
| Ascomycota 2–5                          | 0.387‡   | 1.233     | 0.766          | 0.164          | 0.246    |
| <i>Davidiella</i> / <i>Cladosporium</i> | –1.219   | –0.364    | –0.428         | 0.571          | –0.707   |
| <i>Cryptococcus</i> sp.                 | 0.026    | 0.168     | 0.227          | –0.522         | 0.707    |
| Agaricomycete                           | –0.959   | –0.426    | –0.933         | –0.747         | –1.217   |
| <i>Sistotrema</i> sp.1, sp.2            | –1.011   | –0.655    | –0.44          | –1.149         | –0.603   |

Notes: Larger effect sizes indicate greater differences between the responses of fungi to substrate additions and the control treatments. Different forms of organic N were added to leaf-decaying fungi. Ascomycota 2–5 refers to the pooling of four unidentified species (2, 3, 4, 5).

† From Allison and Treseder (2008).

‡ For both litter types (spruce and aspen),  $n = 6$  in all cases.

FIG. 4. Relationship between degree of response to lignocellulose addition and experimental warming among fungal taxa common to both the current study and a concurrent warming study (Table 1; see Allison and Treseder 2008). Positive effect sizes (Cohen's  $d$ ) indicate that taxa proliferated in response to warming or lignocellulose addition. Negative effect sizes indicate that taxa declined. Warming effect sizes were significantly correlated with lignocellulose effect sizes ( $P = 0.026$ ). The line is best-fit; symbols correspond to the following fungal taxa: solid square, Ascomycota 2–5 (four unidentified species pooled); star, *Cryptococcus* sp.; solid circle, *Sistotrema* spp.; solid diamond, *Davidiella/Cladosporium*; solid triangle, Agaricomycete.



mental warming. Effect sizes for warming were not significantly correlated with effect sizes for any other substrate, nor were they correlated with effect sizes for litter type ( $|r| \leq 0.552$ ). The significant lignocellulose relationship supported our hypothesis (5) that taxa targeting recalcitrant N compounds would respond more positively to warming than would those that did not. However, this hypothesis was only supported with lignocellulose degraders, as the tannin–protein relationship was nonsignificant.

#### DISCUSSION

We found that fungal taxa varied in their use of organic N substrates, suggesting that fungi are functionally diverse in forest soils and that individual taxa can influence the cycling of different organic N substrates. In some cases, multiple taxa of fungi responded similarly to the addition of one substrate, but varied in their response to other substrates. This observation implies that losing individual fungal taxa from the soil could alter organic N cycling because other fungi in the ecosystem may not be able to replace the suite of functions performed by a specific taxon. Differences among taxa in substrate use profiles were linked to genetic relatedness, which may enable researchers to predict ecosystem functions from phylogenies of fungi in soils. Furthermore, experimental warming favored taxa that used lignocellulose, indicating that the differential effects of global change on fungal species may have significant consequences for decomposition and CO<sub>2</sub> release to the atmosphere. Litter type also affected fungal community composition, but not necessarily owing to substrate use traits. In addition, taxa tended to co-occur less frequently than would be expected by chance, suggesting competition among taxa or resource partitioning.

Substrate preferences could be one mechanism underlying diversity–decomposition relationships documented in microcosm studies. Recent studies have manipulated diversity of microbial taxa under laboratory conditions to test for effects on ecosystem functions such as decomposition (e.g., van der Heijden et al. 1998, Naeem

et al. 2000, Setälä and McLean 2004). Typically, ecosystem function increases linearly as the first few taxa are added, but then begins to plateau at higher richness levels. LeBauer (2008) found similar relationships between fungal species richness and CO<sub>2</sub> efflux and cellulase activity in microcosms inoculated with fungi isolated from our field site. A valuable next step would be to directly manipulate fungal communities in natural ecosystems, perhaps through the use of “microbial cages” such as those developed for marsh bacteria by Reed and Martiny (2007).

In the meanwhile, it may be possible to use environmental DNA sequences to loosely predict the decomposer function of active fungal communities, given that closely related taxa in our study were more likely to possess similar patterns of substrate use than were more distantly related taxa. The correlation between genotypic and phenotypic divergence is a fundamental paradigm in evolutionary biology (Darnval et al. 1987, Scharloo 1991). Other studies have documented relationships between genetic and phenotypic similarity for microbial and plant traits (Jasienski et al. 1997, Horz et al. 2005, Borba et al. 2008). To our knowledge, none has examined phenotypes related to decomposition, although the issue has received recent attention (Andren and Balandreau 1999, Balser et al. 2002). A fair degree of scatter occurred within the observed genotypic–phenotypic relationship in our study, which could have resulted from some degree of canalization, independence between the sequencing region and the relevant functional or regulatory genes, or limitations in our methods. Thus, before this information can realistically be incorporated into models, more detailed studies are required to determine which phenotypes of substrate use patterns are more genetically conserved. The BrdU technique could facilitate advancements in our knowledge of these relationships by examining substrate-use phenotypes across a more broad range of litter types with varying degrees of recalcitrance.

Active fungal communities were phylogenetically clustered in response to some substrate additions in aspen

litter, which is additional evidence suggesting that specific substrate-use patterns may be genetically correlated. However, there were no patterns of phylogenetic clustering in spruce litter in relation to substrate use, which may be due to the fact that only certain fungi can persist on spruce needles (Allison et al. 2009). Taxa also differed in abundance and phylogenetic structure on the different litter types, as is commonly observed (Koide et al. 1998, Gebauer and Taylor 1999, Conn and Dighton 2000).

Active fungal communities were not structured randomly within samples; negative relationships among taxa were significantly more common than expected by chance. Moreover, this pattern was evident whether or not the substrate addition treatments were included in the analysis. A number of mechanisms could elicit negative relationships, including competition for limiting resources, habitat specialization, allelopathy, or resource partitioning (Diamond 1975). In our study, the observed variation among taxa in responses to organic N substrates is consistent with resource partitioning, but we cannot rule out the contribution of other mechanisms.

Substrate use traits were often positively correlated with one another within taxa, meaning that a taxon that responded to one substrate also tended to respond similarly to other substrates. The two substrate use traits most strongly correlated with one another were glutamate and arginine. Fungal amino acid transporters have been identified that are capable of taking up both of these amino acids (Nehls et al. 1999, Wipf et al. 2002), which would provide a physiological mechanism for these traits to coevolve. Only four taxa responded significantly to either of the amino acids, which was unexpected given that amino acids are relatively labile sources of N. Fungal-to-bacterial ratios increased following substrate additions, which suggested that bacteria were not competitively inhibiting fungal uptake of amino acids, possibly due to low litter pH, which would favor fungi over bacteria (Fierer and Jackson 2006). Other components of litter chemistry such as polyphenols may have limited the use of amino acids.

The phylogenetic distributions of fungi were different across litter types and substrates, indicating that multiple processes are occurring simultaneously to structure fungal communities. At the level of the substrate, fungi displaying similar substrate preferences were correlated genetically. However, at finer scales when both litter and substrate were separated, both clustering and overdispersion were occurring; in aspen litter, there was more phylogenetic clustering observed within substrates, whereas in spruce litter there was more dispersion. These disparate patterns may be due to the fact that spruce litter is a harsh environment for many fungi, which could preliminarily filter the fungal community. If the fungi that persist on spruce needles are closely related and have similar ecological resource requirements, then competitive exclusion could result in the coexistence of only distantly related taxa (Webb et

al. 2002). At the level of the landscape, these complex interactions of traits, phylogeny, and community structure may be obscured. Thus, the scale at which the resource dimension is studied will determine the detection of phylogenetic community structure, emphasizing the need to interpret seemingly random patterns with caution.

Biases are inherent in every current method of characterizing microbial communities in the soil, and BrdU labeling is no exception. Fungal groups may have differed in rates of BrdU incorporation, and not all taxa may have possessed the capacity to take up BrdU (Urbach et al. 1999, Pernthaler et al. 2002). Nevertheless, a previous study in our field site found no substantial differences in fungal community composition between BrdU-labeled and unlabeled DNA fractions extracted from the same soil samples (collected from both control and long-term N-fertilized plots [Allison et al. 2007]), which indicates that BrdU uptake occurs broadly among local fungal taxa. Furthermore, we quantified responses of fungal taxa to substrate additions relative to a control. This approach may have partially compensated for variation among groups in baseline rates of BrdU uptake. Another compromise was incubation time. The 48-hour incubation was chosen to avoid cross-feeding, but with a consequence of selecting for taxa that could respond relatively quickly to substrate enrichment (Harder and Dijkhuizen 1982, Dunbar et al. 1997). It is possible that incubations were too short to allow users of recalcitrant substrates (especially tannin-protein complexes) to proliferate. Furthermore, cross-feeding may be more likely with labile substrates.

### Conclusion

Fungal taxa in an Alaskan boreal forest varied in preferences for organic N substrates, and this variation was related to phylogeny. Moreover, the degree of response by taxa to lignocellulose addition was positively correlated with the degree of response to experimental warming. Our results suggest that fungal community composition can play a role in organic N dynamics in ecosystems, and that shifts in communities under global warming could have consequences for breakdown of lignocellulose, a common macromolecule in plant litter. Knowledge of fungal community effects on decomposition could improve our predictions of nutrient cycling under global change, and our finding of a relationship between phylogeny and organic N use may facilitate this approach.

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## LITERATURE CITED

- Abuzinadah, R. A., and D. J. Read. 1986. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. 1. Utilization of peptides and proteins by ectomycorrhizal fungi. *New Phytologist* 103:481–493.
- Allison, S. D., C. I. Czimczik, and K. K. Treseder. 2008. Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. *Global Change Biology* 14:1156–1168.
- Allison, S. D., C. A. Hanson, and K. K. Treseder. 2007. Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. *Soil Biology and Biochemistry* 39:1878–1887.
- Allison, S. D., D. S. LeBauer, M. R. Ofrecio, R. Reyes, A. M. Ta, and T. M. Tran. 2009. Low levels of nitrogen addition stimulate decomposition by boreal forest fungi. *Soil Biology and Biochemistry* 41:293–302.
- Allison, S. D., and J. B. H. Martiny. 2008. Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences (USA)* 105:11512–11519.
- Allison, S. D., and K. K. Treseder. 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Global Change Biology* 14:2898–2909.
- Allison, S. D., and P. M. Vitousek. 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biology and Biochemistry* 37:937–944.
- Andren, O., and J. Balandreau. 1999. Biodiversity and soil functioning—from black box to can of worms? *Applied Soil Ecology* 13:105–108.
- Balser, T. C., A. P. Kinzig, and M. K. Firestone. 2002. Linking soil communities and ecosystem functioning. Pages 265–293 in A. P. Kinzig, S. W. Pacala, and D. Tilman, editors. *The functional consequence of biodiversity: empirical progress and theoretical extensions*. Princeton University Press, Princeton, New Jersey, USA.
- Bent, E., B. Yin, A. Figueroa, J. X. Ye, Q. Fu, Z. Liu, V. McDonald, D. Jeske, T. Jiang, and J. Borneman. 2006. Development of a 9600-clone procedure for oligonucleotide fingerprinting of rRNA genes: utilization to identify soil bacterial rRNA genes that correlate in abundance with the development of avocado root rot. *Journal of Microbiological Methods* 67:171–180.
- Bergner, B., J. Johnstone, and K. K. Treseder. 2004. Experimental warming and burn severity alter soil CO<sub>2</sub> flux and soil functional groups in a recently burned boreal forest. *Global Change Biology* 10:1996–2004.
- Borba, C. D., J. Correia, E. Vinhas, A. Martins, B. C. A. Alves, S. Unkles, J. R. Kinghorn, and N. Lucena-Silva. 2008. Genetic characterization of morphologically variant strains of *Paracoccidioides brasiliensis*. *Memorias Do Instituto Oswaldo Cruz* 103:306–309.
- Borneman, J. 1999. Culture-independent identification of microorganisms that respond to specified stimuli. *Applied and Environmental Microbiology* 65:3398–3400.
- Borneman, J., and R. J. Hartin. 2000. PCR primers that amplify fungal rRNA genes from environmental samples. *Applied and Environmental Microbiology* 66:4356–4360.
- Cairney, J. W. G. 1999. Intraspecific physiological variation: implications for understanding functional diversity in ectomycorrhizal fungi. *Mycorrhiza* 9:125–135.
- Chenna, R., H. Sugawara, T. Koike, R. Lopez, T. J. Gibson, D. G. Higgins, and J. D. Thompson. 2003. Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Research* 31:3497–3500.
- Cohen, J. 1992. A power primer. *Psychological Bulletin* 112:155–159.
- Conn, C., and J. Dighton. 2000. Litter quality influences on decomposition, ectomycorrhizal community structure and mycorrhizal root surface acid phosphatase activity. *Soil Biology and Biochemistry* 32:489–496.
- Crawford, D. L., and R. L. Crawford. 1976. Microbial degradation of lignocellulose: the lignin component. *Applied and Environmental Microbiology* 31:714–717.
- Crawford, D. L., R. L. Crawford, and A. L. Pometto. 1977. Preparation of specifically labeled C-14-(lignin)-lignocelluloses and C-14-(cellulose)-lignocelluloses and their decomposition by microflora of soil. *Applied and Environmental Microbiology* 33:1247–1251.
- Damerval, C., Y. Hebert, and D. Devienne. 1987. Is the polymorphism of protein amounts related to phenotypic variability? A comparison of two-dimensional electrophoresis data with morphological traits in maize. *Theoretical and Applied Genetics* 74:194–202.
- Davidson, E. A., and I. A. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440:165–173.
- Diamond, J. M. 1975. Assembly of species communities. Pages 342–444 in M. L. Cody and J. M. Diamond, editors. *Ecology and evolution of communities*. Harvard University Press, Cambridge, Massachusetts, USA.
- Dunbar, J., S. White, and L. Forney. 1997. Genetic diversity through the looking glass: effect of enrichment bias. *Applied and Environmental Microbiology* 63:1326–1331.
- Durall, D. M., A. W. Todd, and J. M. Trappe. 1994. Decomposition of C-14-labeled substrates by ectomycorrhizal fungi in association with Douglas-fir. *New Phytologist* 127:725–729.
- Fierer, N., and R. B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences (USA)* 103:626–631.
- Fierer, N., J. A. Jackson, R. Vilgalys, and R. B. Jackson. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental Microbiology* 71:4117–4120.
- Frey, S. D., M. Knorr, J. L. Parrent, and R. T. Simpson. 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *Forest Ecology and Management* 196:159–171.
- Gebauer, G., and A. F. S. Taylor. 1999. N-15 natural abundance in fruit bodies of different functional groups of fungi in relation to substrate utilization. *New Phytologist* 142:93–101.
- Gotelli, N. J., and G. L. Entsminger. 2001. EcoSim: null models software for ecology. Acquired Intelligence and Kesey-Bear, Montrose, Colorado, USA.
- Hanson, C. A., S. D. Allison, M. A. Bradford, M. D. Wallenstein, and K. K. Treseder. 2008. Fungal taxa target different carbon sources in forest soil. *Ecosystems* 11:1157–1167.
- Harder, W., and L. Dijkhuizen. 1982. Strategies of mixed substrate utilization in microorganisms. *Philosophical Transactions of the Royal Society B* 297:459–480.
- Haselwandter, K., O. Bobleter, and D. J. Read. 1990. Degradation of C-14-labeled lignin and dehydropolymer of coniferyl alcohol by ericoid and ectomycorrhizal fungi. *Archives of Microbiology* 153:352–354.
- Horton, T. R., and T. D. Bruns. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology* 10:1855–1871.
- Horz, H. P., V. Rich, S. Avrahami, and B. J. M. Bohannan. 2005. Methane-oxidizing bacteria in a California upland grassland soil: diversity and response to simulated global change. *Applied and Environmental Microbiology* 71:2642–2652.
- Jasienski, M., F. J. Ayala, and F. A. Bazzaz. 1997. Phenotypic plasticity and similarity of DNA among genotypes of an annual plant. *Heredity* 78:176–181.

- Kasischke, E. S., and B. J. Stocks. 2000. Fire, climate change, and carbon cycling in the boreal forest. Springer, New York, New York, USA.
- Koide, R. T., L. Suomi, C. M. Stevens, and L. McCormick. 1998. Interactions between needles of *Pinus resinosa* and ectomycorrhizal fungi. *New Phytologist* 140:539–547.
- LeBauer, D. S. 2008. Carbon balance of ecosystems under nitrogen enrichment. Dissertation. University of California, Irvine, California, USA.
- Liedoff, A. C. 1999. Mantel nonparametric test calculator. School of Natural Resource Sciences, Queensland University of Technology, Queensland, Australia.
- Lilleskov, E. A., T. J. Fahey, T. R. Horton, and G. M. Lovett. 2002. Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. *Ecology* 83:104–115.
- Mantel, N. 1967. Detection of disease clustering and a generalized regression approach. *Cancer Research* 27:209–220.
- Milliken, G. A., and D. E. Johnson. 2002. Analysis of messy data. Chapman and Hall/CRC, New York, New York, USA.
- Naeem, S., D. R. Hahn, and G. Schuurman. 2000. Producer–decomposer co-dependency influences biodiversity effects. *Nature* 403:762–764.
- Nehls, U., R. Kleber, J. Wiese, and R. Hampp. 1999. Isolation and characterization of a general amino acid permease from the ectomycorrhizal fungus *Amanita muscaria*. *New Phytologist* 144:343–349.
- Pace, N. R. 1997. A molecular view of microbial diversity and the biosphere. *Science* 276:734–740.
- Pernthaler, A., J. Pernthaler, M. Schattnerhofer, and R. Amann. 2002. Identification of DNA-synthesizing bacterial cells in coastal North Sea plankton. *Applied and Environmental Microbiology* 68:5728–5736.
- Reed, H. E., and J. B. H. Martiny. 2007. Testing the functional significance of microbial composition in natural communities. *FEMS Microbiology Ecology* 62:161–170.
- Sanderson, M. A., and W. F. Wedin. 1989. Nitrogen in the detergent fiber fractions of temperate legumes and grasses. *Grass and Forage Science* 44:159–168.
- Scharloo, W. 1991. Canalization: genetic and developmental aspects. *Annual Review of Ecology and Systematics* 22:65–93.
- Schimel, J. P., J. Bennett, and N. Fierer. 2004. Microbial community composition and soil N cycling: Is there really a connection? Pages 1–24 in *Annual Symposium: Soil Biodiversity and Function*. British Ecological Society, Lancaster, UK.
- Schloss, P. D., and J. Handelsman. 2005. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Applied and Environmental Microbiology* 71:1501–1506.
- Setälä, H., and M. A. McLean. 2004. Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi. *Oecologia* 139:98–107.
- SPSS. 2002. Systat version 10 for Windows. SPSS, Chicago, Illinois, USA.
- Stevenson, F. J. 1994. Humus chemistry. Genesis, composition, reactions. John Wiley and Sons, New York, New York, USA.
- Stone, L., and A. Roberts. 1990. The checkerboard score and species distributions. *Oecologia* 85:74–79.
- Stouthammer, A. H. 1973. A theoretical study of the amount of ATP required for the microbial cell material. *Antonie van Leeuwenhoek* 39:545–565.
- Treseder, K. K. 2005. Nutrient acquisition strategies of fungi and their relation to elevated atmospheric CO<sub>2</sub>. Pages 713–731 in J. Dighton, P. Oudemans, and J. White, editors. *The fungal community*. CRC Press, Boca Raton, Florida, USA.
- Treseder, K. K., M. C. Mack, and A. Cross. 2004. Relationships among fires, fungi, and soil dynamics in Alaskan boreal forests. *Ecological Applications* 14:1826–1838.
- Tuomela, M., P. Oivanen, and A. Hatakka. 2002. Degradation of synthetic C-14-lignin by various white-rot fungi in soil. *Soil Biology and Biochemistry* 34:1613–1620.
- Urbach, E., K. L. Vergin, and S. J. Giovannoni. 1999. Immunochemical detection and isolation of DNA from metabolically active bacteria. *Applied and Environmental Microbiology* 65:1207–1213.
- Valinsky, L., G. Della Vedova, T. Jiang, and J. Borneman. 2002a. Oligonucleotide fingerprinting of rRNA genes for analysis of fungal community composition. *Applied and Environmental Microbiology* 68:5999–6004.
- Valinsky, L., G. Della Vedova, A. J. Scupham, S. Alvey, A. Figueroa, B. Yin, J. Hartin, M. Chrobak, D. E. Crowley, T. Jiang, and J. Borneman. 2002b. Analysis of bacterial community composition by oligonucleotide fingerprinting of rRNA genes. *Applied and Environmental Microbiology* 68:3243–3250.
- van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglou, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396:69–72.
- Villani, F., M. Pigliucci, M. Lauteri, and M. Cherubini. 1992. Congruence between genetic, morphometric, and physiological data on differentiation of Turkish chestnut (*Castanea sativa*). *Genome* 35:251–256.
- Webb, C. O., D. D. Ackerly, M. A. McPeck, and M. J. Donoghue. 2002. Phylogenies and community ecology. *Annual Review of Ecology and Systematics* 33:475–505.
- Webb, C. O., and M. J. Donoghue. 2005. Phylomatic: tree assembly for applied phylogenetics. *Molecular Ecology Notes* 5:181–183.
- Wipf, D., M. Benjdia, M. Tegeder, and W. B. Frommer. 2002. Characterization of a general amino acid permease from *Hebeloma cylindrosporum*. *FEBS Letters* 528:119–124.
- Yeates, C., and M. R. Gillings. 1998. Rapid purification of DNA from soil for molecular biodiversity analysis. *Letters in Applied Microbiology* 27:49–53.
- Yin, B., L. Valinsky, X. B. Gao, J. O. Becker, and J. Borneman. 2003. Identification of fungal rDNA associated with soil suppressiveness against *Heterodera schachtii* using oligonucleotide fingerprinting. *Phytopathology* 93:1006–1013.

## APPENDIX A

Additional methods used for DNA fingerprinting, substrate preparations, quantitative PCR, and data analysis (*Ecological Archives* E091-165-A1).

## APPENDIX B

Relative proportions and phylogenetic relationships of the most abundant clones detected in response to substrate additions and litter type (*Ecological Archives* E091-165-A2).